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厦门大学

硕　士　学　位　论　文

辣根过氧化物酶催化合成 PEDOT 及其应用

Conductive Poly（3,4-ethylenedioxythiophene） Synthesized by Horseradish Peroxidase and its Application

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摘要

导电高分子聚二氧乙撑噻吩（PEDOT, poly-（3,4-ethylenedioxythiophene））近年来引起了很多人研究兴趣，主要是由于 PEDOT 具有较低的能带隙、优良的导电性（10~10^5 S/cm）、显著的电致变色特性以及环境热稳定性，成为当前引人注目的商业化应用型导电聚合物。

目前常用的化学法聚合聚二氧乙撑噻吩/聚苯乙烯磺酸盐（（poly-（3,4-ethylenedioxythiophene）/poly（styrenesulfonate），PEDOT/PSS），由于使用大量的强酸和极强的氧化剂，不仅回收处理困难并且污染环境，所以本研究采用辣根过氧化物酶（Horseradish peroxidase, HRP）作为催化剂，配合氧化剂 H_2O_2，以期开发一种对环境更加温和、友好，应用于生物材料的 PEDOT/PSS 薄膜。本研究主要包括的内容和结果如下：

一、构建了 HRP 催化合成 PEDOT/PSS 的合成体系，通过考察 HRP 在不同 pH（2~7）、不同过氧化氢（H_2O_2）和十二烷基硫酸钠（Sodium dodecyl sulfate, SDS）浓度下的活性变化，建立了 HRP 最佳的反应条件。再结合 HRP 催化合成 PEDOT/PSS 的条件验证对比，最终确定 PEDOT/PSS 合成的最优条件，即，缓冲液为 200 mM 的 Citric acid-phosphate（pH 2），反应温度 25^{o}C。反应体系中 SDS 浓度为 0 mM，而 H_2O_2 则分 6 次添加，每次添加浓度不超过 4 mM，终浓度为 25 mM。

二、本研究验证了加热与模板 PSS 在合成中的重要作用，建立 HRP 两步法合成 PEDOT/PSS，提出并验证了两步法合成的机理，即，HRP 在第一步中将 EDOT 单体转变为 EDOT 自由基，并在模板 PSS 的存在下连成寡聚物；第二步加热使得链继续延伸。通过紫外分光光度计（Ultraviolet-visible spectrophotometer, UV）初步检测并探讨其中关键因素 HRP 浓度、反应时间、加热温度、加热时间对合成的影响，建立了完整的 HRP 合成 PEDOT/PSS 的条件，即，HRP 浓度为 0.5 mg/mL，反应时间 16 h，在 60 ^{o}C 加热 150 min 后得到产物 PEDOT/PSS。

三、通过比较 16 种不同有机溶剂（乙腈、甲基叔丁基醚、丙酮、乙酸酐、甲基异丙酮、吡啶、氯丁烷、乙醇、甲苯、己烷、二乙醚、异丙醇、甲醇、正丁醇、二甲基亚砜、氯仿）对 PEDOT/PSS 的萃取效果，最终选取正丁醇作为纯化

溶剂。将纯化后的 PEDOT/PSS 用霍式转换红外线光谱（FT-IR）、热重分析仪（TGA）、场发式扫描电子显微镜（SEM）、共振拉曼光谱仪（Raman）、高解析电子能谱仪（XPS）、核磁共振波谱仪（NMR）表征，并用四点探针和循环伏安法进行电化学性质分析，证明了 HRP 合成的 PEDOT/PSS 颗粒细致，符合 quinol 结构，与模板 PSS 结合力强，还具有良好的电化学活性，PEDOT/PSS 与石墨以重量比 4:1 掺杂混合后的导电性为 12.84 S/cm。

四、将生物相容性高分子材料 PVA 与 PEDOT/PSS 混合，并通过电仿和涂布的方式制膜，比较了膜的均匀性和导电性，最终确定以涂布的方式制膜。用 SEM 和 XRD 观察 PEDOT-PVA 表面形态，证明涂布的 PEDOT-PVA 膜平整细致，并考察了骨细胞 MG 63 对 PEDOT-PVA 膜培养情况的应答，证明骨细胞能很好的在膜上生长，并为后期细胞在电刺激作用下修复生长提供了基础。

关键词：辣根过氧化物酶 导电高分子 PEDOT/PSS 过氧化氢

Abstract

The conductive polymer, poly-（3,4-ethylenedioxythiophene）（PEDOT）, has drawn much attention in recent years due to the features of its low band-gap, great conductivity, pronounced electrochromic activity, and long-term stability. PEDOT has shown great promise as one of the most feasible and commercial inherently conductive polymers（ICP）.

At present, PEDOT is commonly synthesized by chemical approaches, however, a large number of strong acids and strong oxidants are normally required, which pollute the environment and the products are lack of good processibility. Nevertheless, Horseradish peroxidase（HRP）synthesis of PEDOT, with hydrogen peroxide as oxidant, has been promoted as the idea of green chemistry since HRP allow PEDOT be synthesized in milder reaction conditions, which provide environmentally friend processes. Our research focused on PEDOT/PSS, which applied to biomaterial this new field. Major works were listed as follow:

The primary HRP catalyzing reaction was set up by optimizing key factors, including pH from 2 to 7, different Sodium dodecyl sulfate（SDS）, and H_2O_2 concentration. That, combined with the PEDOT/PSS synthesized by HRP, eventually confirmed the best HRP polymerization was obtained in 200 mM Citric acid-phosphate（pH 2）buffer at 25^{o}C, where the SDS concentration was 0 mM, and the H_2O_2, which was appended by many times, each time was lower than 4 mM, ultimate concentration was 25 mM.

In this study, we have proposed a two-stage procedure for the enzymatic synthesized of PEDOT, and then led heating into synthesis. Meanwhile, we further evaluated the importance of PSS as both template and doping agent, and heating procedure have exhibited the significant contribution to PEDOT synthesis. Accordingly, weproposed and validated the mechanism in this two-stage method, where HRP acts as the catalyst to promote the generation of EDOT free radicals followed by the oligomerization under the room temperature in the presence of PSS, then a mild heating process is employed for the chain extension. A complete HRP catalysis PEDOT/PSS system was established by studying key factor, including HRP concentration, reaction time, heating temperature and time. The reaction was finally carried out with 0.5 mg/mL HRP, after 16 h reaction and heating 150 mins at 60 ^{o}C.

Compared the extraction rate of 16 different organic solvent to PEDOT/PSS, including acetonitrile, methyl t-butyl ether, acetone, acetic anhydride, methyl iso-butyl ketone, pyridine, 1-chlorobutane, ethanol, toluene, hexanes, diethyl ether, isopropyl alcohol, methanol, n-butanol, DMSO, chloroform, we finally considered n-butanol the best extraction organic solvent. After purification of PEDOT/PSS, morphologies of which were characterized by scanning electron microscope（SEM）, the molecular structures of which was certificated by fourier transform infranred spectrometer（FT-IR）, thermo gravimetric analyzer（TGA）, Raman, Nuclear Magnetic Resonance（NMR）, High resolution X-ray Photoelectron Spectrometer（XPS）, conductivities and electrochemical properties of which were characterized by the four-probe technology and cyclic voltammetry as well. All these verified that PEDOT/PSS, for enzymatic synthesized, was fine-grained particle, quinol structure, and strong bound with PSS. Besides, it possessed an excellent electrochemical properties, and the average conductivity of PEDOT: graphite composite（weight ratio = 4:1）at room temperature was approximately 12.84 S/cm.

While PEDOT/PSS have high conductivity when not blended with biocompatible PVA, they are brittle and difficult to coat or electrospin. Thus, the film was made with PEDOT blended with PVA by coating based on the dispersible uniformity and conductivity. SEM and XRD were employed to discuss the morphologies of the film and suggested fine and smooth. In addition, the response of bone cells MG 63 to PEDOT-PVA film was investigated based on their growth on the film, which was detected with MTT and SEM, demonstrated that the cells grew well on the film.

Key words: Horseradish peroxidase（HRP） conductive polymer PEDOT/PSS hydrogen peroxide

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